

WHAT IS CLAIMED IS:

1 1. A method of mapping the location of a post-translational modification
2 of a post-translationally modified peptide, said method comprising:

3 (a) contacting said peptide with a chemical modification reagent that converts
4 a post-translationally modified amino acid residue of said peptide into a substrate for a
5 peptidase, thereby producing a chemically modified peptide comprising a chemically
6 modified amino acid residue;

7 (b) contacting said chemically modified peptide with said peptidase under
8 conditions appropriate to degrade said chemically modified peptide, thereby producing a
9 degraded chemically modified peptide; and

10 (c) querying said degraded chemically modified peptide to ascertain said
11 location of said post-translational modification.

1 2. The method of claim 1, further comprising:

2 (d) prior to step (a), contacting a substrate amino acid of said peptide that is a
3 natural substrate for said peptidase with a blocking agent thereby converting said substrate
4 amino acid into a side-chain protected amino acid that is not a substrate for said peptidase.

1 3. The method of claim 2, wherein said substrate amino acid is a lysine,
2 wherein said blocking agent converts said lysine into a side-chain protected lysine selected
3 from the group consisting of a carbamate, an amide, an N-sulfonyl, an N-sulfenyl, an N-nitro,
4 an N-nitroso, an N-oxide, an imine, an N-alkyl amine, an N-aryl amine, an N-phosphinyl, an
5 N-phosphoryl, and an enamine .

1 4. The method of claim 2, wherein said side chain protected lysine is
2 selected from the group consisting of Lys(Aloc), Lys(Ac), Lys(Boc), Lys(biotinyl), Lys(2-
3 bromo-Z), Lys(2-chloro-Z), Lys(Dnp), Lys(Fmoc), Lys(For), Lys(Me)₂, Lys(nicatinoyl),
4 Lys(Tfa), Lys(Tos), Lys(Z), Lys(Z)(isopropyl), Lys(Boc)(isopropyl), Lys(dansyl), Lys(Dde),
5 Lys(Me)₃, Lys(Mtt), Lys(palitoyl), Lys(TNM), Lys(acetimidoyl), Lys(2,4,-dichloro-Z),
6 Lys(Me), Lys(p-nitro-Z), Lys(5/6 FAM), Lys(pyrenebutyryl), and Lys(guanidinyl).

1 5. The method of claim 1, wherein said substrate amino acid is aspartic
2 acid, wherein said blocking agent converts said aspartic acid into a side-chain protected
3 aspartic acid selected from an ester, an amide, an oxalose, an oxazolines, a stannyl ester, and
4 an hydrazide.

1 6. The method of claim 1, wherein side chain protected aspartic acid is
2 selected from the group consisting of Asp(OBzl), Asp(OcHex), Asp(OtBu), Asp(OMpe),
3 Asp(Ofm), Asp(Osu), Asp(2-phenylisopropyl ester), and Asp(ONp).

1 7. The method of claim 1, wherein said peptidase is selected from the
2 group consisting of a serine endopeptidase, a metalloendopeptidase, a cysteine
3 endopeptidase, and an aspartic endopeptidase.

1 8. The method of claim 1, wherein said peptidase is a lysine-specific
2 peptidase.

1 9. The method of claim 8, wherein said lysine-specific peptidase is
2 selected from the group consisting of endoproteinase Lys-C, lysyl endopeptidase, trypsin,
3 plasma kallikrein, oligopeptidase B, tryptase, plasmin, acrosin, granzyme A, yapsin 1,
4 peptidyl-Lys metalloendopeptidase, and magnolsyin.

1 10. The method of claim 8, wherein said lysine-specific peptidase is
2 selected from the group consisting of endoproteinase Lys-C, lysyl endopeptidase and trypsin.

1 11. The method of claim 1, wherein said peptidase is an aspartate-specific
2 peptidase.

1 12. The method of claim 11, wherein said aspartate-specific peptidase is
2 selected from peptidyl-aspartate metalloendopeptidase and nepenthesin.

1 13. The method of claim 1, wherein said querying comprises mass
2 spectrographic detection of said chemically modified amino acid residue of said degraded
3 chemically modified peptide.

1 14. The method according to claim 1, further comprising:
2 (e) prior to step (a), contacting said peptide with an elimination reagent that
3 causes the elimination of a post-translationally added substituent of said post-translationally
4 modified amino acid residue.

1 15. The method of claim 14, wherein said post-translationally modified
2 amino acid residue is selected from the group consisting of a post-translationally modified
3 serine and a post-translationally modified threonine.

1 16. The method of claim 14, wherein said post-translationally modified
2 amino acid residue is a phosphorylated amino acid residue.

1 17. The method according to claim 14, wherein said elimination is a β -
2 elimination giving rise to an alkene moiety.

1 18. The method according to claim 1, wherein said modification reagent
2 reacts with said post-translationally modified amino acid residue via a Michael addition.

1 19. The method of claim 18, wherein said modification reagent is selected
2 from the group consisting of sodium sulfate and cysteamine.

1 20. A reactive solid phase material comprising:
2 (a) a solid support; and
3 (b) a solid support reactive moiety immobilized on said solid support, wherein
4 said solid support reactive moiety is reactive towards a synthetically modified amino acid
5 residue of a post-translationally modified peptide, said synthetically modified amino acid
6 residue produced by elimination a post-translationally added substituent of said post-
7 translationally modified peptide.

1 21. The material according to claim 20, wherein said synthetically
2 modified amino acid residue comprises an alkene moiety.

1 22. A method of immobilizing a post-translationally modified peptide
2 comprising a post-translationally modified amino acid, said method comprising:
3 (i) contacting said peptide with an elimination reagent that causes the
4 elimination of a post-translationally added substituent of said post-translationally modified
5 amino acid residue thereby producing a synthetically modified amino acid;
6 (ii) reacting said synthetically modified amino acid with a reactive solid phase
7 material thereby immobilizing said post-translationally modified peptide, said reactive solid
8 phase material comprising:
9 (a) a solid support; and
10 (b) a solid support reactive moiety immobilized on said solid support,
11 wherein said solid support reactive moiety is reactive towards said
12 synthetically modified amino acid residue.